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Isospongiadiol, a Cytotoxic and Antiviral Diterpene from a Caribbean Deep Water Marine Sponge, Spongia sp.

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Spongiadiol, epispongiadiol, and isospongiadiol, a novel furanoditerpene with cytotoxic and antiviral activity, were isolated from a Caribbean deep water marine sponge, Spongia sp. Isospongiadiol was identified by spectral and chemical methods as a regioisomer of spongiadiol and epispongiadiol.

During one of our ship-based collecting efforts to identify biologically active compounds from marine organisms, we discovered that an extract from a deepwater Caribbean sponge, Spongia sp. Linnaeus, 1759,[1] exhibited activity against Herpes simplex virus, type 1 (HSV-1), and P388 murine leukemia cells. Subsequent purification yielded three active compounds, spongiadiol (1), epispongiadiol (2) and a structurally related compound, which we have designated isospongiadiol (2α,19-dihydroxyspongia-13(16),14-dien-3-one) (3). Spongiadiol and epispongiadiol, tetracyclic furanoditerpenes with the spongian ring system, have been identified previously from Spongia species collected in Australian waters.[2] In this note, we describe the isolation and identification of isospongiadiol (3), and the antiviral and cytotoxic activities of all three compounds.

The sponge was collected adjacent to Chub Cay, Bahamas, in December, 1984, at a depth of 203 m using the Harbor Branch Oceanographic Institution's submersible, the Johnson Sea-Link II. Extraction of the fresh frozen sponge (94 g) with EtOAc provided a crude residue (0.97 g). Repeated multilayer planetary coil CCC [3-4] of a portion (0.55 g) of the crude residue yielded spongiadiol (1, 39 mg, 0.13% of frozen weight), epispongiadiol (2, 263 mg, 0.87%), and isospongiadiol (3, 61 mg, 0.2%). Isospongiadiol (3) was further purified by recrystallization from MeOH/H_2O: mp 181-183 °C, $[\alpha]_D^{20}$ -50° (c 3.0, CH_2Cl_2).

The molecular formula of 3 was established as $C_{20}H_{28}O_4$ by high resolution EIMS (M^+ 332.1980, Δ2.5 mmu), and is identical to the molecular formula for 1 and 2.[5] Similar to 1, 3 exhibited IR bands at 3400 cm^{-1} and 1700 cm^{-1} for hydroxyl and carbonyl functionalities, respectively. The ^1H NMR spectrum of 3 (C_6D_6-$CDCl_3$ (ca. 2:1), 360 MHz) revealed resonances common to 1, 2, and 3: the α-protons of the furan moiety at δ 6.89 (broad s) and 6.87 (broad s,), three methyl singlets at δ 0.86, 0.99, and 1.11, and an AB pattern at δ 3.16 and 3.70 (J=11.0 Hz). In contrast to the ^1H NMR singlets observed for the β and α protons at C-3 in 1 and 2, respectively, a double doublet was observed in the spectrum of 3 at δ 4.40 (dd,

J=6.5,12.6 Hz) suggesting an alternative oxidation pattern in the A ring. The relationships between this proton and vicinal protons were more clearly defined in the ^{1}H NMR spectrum of isospongiadiol diacetate (4)($CDCl_3$).[6] From proton decoupling experiments, the acetoxy methine proton in 4 observed at 5.60 was shown to be coupled to two protons at δ 2.43 and 1.44 by 6.0 and 13.3 Hz, respectively. The protons at δ 2.43 and 1.44 exhibited a geminal coupling constant of 13.9 Hz. To determine whether the hydroxyl and carbonyl functionalities in 3 were located at C-2 and C-3, or C-2 and C-1, respectively, 2D NMR experiments were employed.

A C-H correlation experiment (J=140 Hz)[7] established the relationships between the protons and carbons in 3 (Table 1). A COSY experiment[8] verified the presence of the coupled proton spin system in the A ring and revealed the presence of other coupled resonances (Table 1). A COSY experiment with emphasis on long range coupling (J=3 Hz) established the relationship of several noncontiguous spin systems (Table 1). A C-H correlation experiment with emphasis on three bond C-H coupling (J=10 Hz), established that the carbonyl carbon (δ214.1, now assigned as C-3) was related to one of the protons of the C-19 AB pattern at δ 3.16, and to the C-18 methyl protons at δ1.11, and that the methyl protons assigned to C-20 (δ0.86) were related to the carbon bearing the methylene protons at δ0.90 and 2.36 (now assigned to C-1). A homonuclear 2-D nOe experiment[9] showed dipolar interactions between the hydroxy methine on C-2 and the methyl protons on C-20, and the methyl protons on C-17 and C-20, which, from inspection of molecular models, suggested 1,3-diaxial relationships between these substituents, and, therefore, that ring A is present in the chair conformation and the stereochemistry of the hydroxy methine proton is β. Ring A in 1 and 2 is in the boat conformation.[2]

The ring A oxidation pattern and absolute configuration of 3 was confirmed by comparison of ^{1}H NMR spectra and optical rotations of the reduction products of 1 - 3.[10] Reduction of 1 - 3 with sodium borohydride in MeOH at room temperature yielded the four possible isomeric triols. The major epimer from reduction of 3 yielded an optical rotation and ^{1}H NMR and (low resolution) EI mass spectral patterns identical to those of the minor epimer derived from reduction of 2, which is depicted as 5. Similarly, the minor epimer from reduction of 3 was found to correspond to the minor epimer from 1 and is assigned as 6. As expected, the reduction occurred predominately from the less hindered α face. In conclusion, the absolute configuration of isospongiadiol (3) is the same as that for 1 and 2 with the exception of carbons 2 and 3.

From in vitro assays against P388 cells, spongiadiol (1), epispongiadiol (2), and isospongiadiol (3) yielded IC_{50} values of 0.5, 8, and 5 μg/ml, respectively. (The IC_{50} value for the standard, vinblastine, is 0.01 μg/ml.) Against HSV-1, the IC_{50} values for 1, 2, and 3 were 0.25, 12.5, and 2 μg/ml, respectively. (IC_{50} values for the standards ara-A and acyclovir are 50 and 0.5 μg/ml, respectively.)

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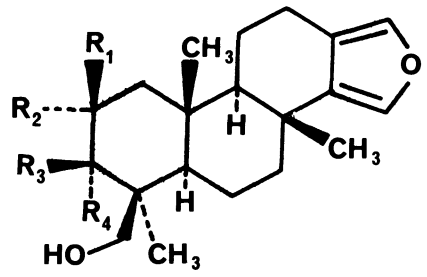

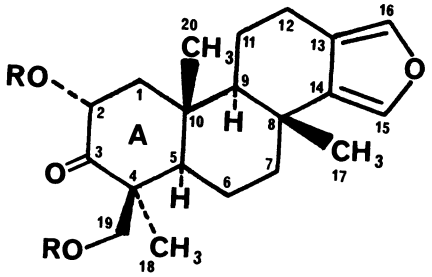

1, R_1+ R_2= O, R_3= H, R_4= OH
2, R_1+ R_2= O, R_3= OH, R_4= H
5, R_1= R_4= H, R_2= R_3= OH
6, R_1= R_3= H, R_2= R_4= OH

3, R = H
4, R = OAc

Table 1. NMR Data of Isospongiadiol (3) in C_6D_6 - $CDCl_3$ (2:1)

C No.- δC	δH[a] at C (m, J(Hz))	Long-range C-H correlations	Long-range H-H correlations	nOe
1-49.8(t)	a-0.90(dd,J=12.6,12.6)	H20	-	-
	b-2.36(dd,J=12.6,6.5)	H20	-	-
2-70.0(d)	4.40(dd,J=12.6,6.5)	-	H19b	H20
3-214.1(s)	-	H18,19b	-	-
4-54.6(s)	-	H18	-	-
5-58.4(d)	0.95(m)	H7a,18,20	-	-
6-20.0(t)	1.28(m,2H)	H7b	-	-
7-41.1(t)	a-1.77(br.d.,J=12.0)	H17	H5	-
	b-1.19(m)	-	-	-
8-34.3(s)	-	H17	-	-
9-55.5(d)	0.80(br.d.,J=11.4)	H7a,17,20	H11a	-
10-37.9(s)	-	H6,20	-	-
11-18.9(t)	a-1.45(m)	H9	-	-
	b-1.37(m)	-	-	-
12-20.6(t)	a-2.50(dd,J=16.1,6.1)	-	H16	-
	b-2.19(m)	-	H16	-
13-119.5(s)	-	-	-	-
14-136.9(s)	-	-	-	-
15-135.2(d)	6.89(br.s)	-	-	-
16-137.2(d)	6.87(br.s)	-	H12a,12b	-
17-26.4(q)	0.99(s) (3H)	H9	-	-
18-19.4(q)	1.11(s) (3H)	H19a	H19a	H19b
19-65.5(t)	a-3.70(d,J=11.0)	-	H18	H20
	b-3.16(d,J=11.0)	-	H2	H18
20-17.5(q)	0.86(s) (3H)	-	H5	H2, 17, 19a

a) a and b are for H-H, C-H, and nOe correlations only, not stereochemistry.

References

1) The tentative identification of the sponge was made by S. Pomponi per the description for Spongia Linnaeus, 1759; family Spongiidae Gray, 1867.

2) R. Kazlauskas, P.T. Murphy, R.J. Wells, K. Noack, W.E. Oberhansli, and P. Schonholzer, Aust. J. Chem., 32, 867 (1979).

3) Y. Ito, J. Sandlin, and W.G. Bowers, J. Chromatogr., 244, 247 (1982).

4) Chromatographic system - Ito multi-layer coil separator-extractor (P.C., Inc.). Solvent system - EtOAc-heptane-MeOH-H_2O (7:4:4:3). The upper phase was used as the mobile phase at a flow rate of 4 ml/min. The instrument was operated at 800 rpm.

5) Spectral data for spongiadiol (1) and epispongiadiol (2) were identical in all respects to published values.[2)]

6) For 4: colorless oil, $[\alpha]_D^{20}$ -32° (c 3.4, $CHCl_3$), HREIMS m/z 416.2198, $C_{24}H_{32}O_6$, Δ-0.6 mmu, IR (film): 1730, 1230 cm^{-1}, ^1H NMR (360 MHz, $CDCl_3$): selected peaks, δ7.06 (d, J=1.5, 1H), 7.03 (d, J=1.3, 1H), 5.60 (dd, J=6.0, 13.3, 1H), 4.74 (d, J=11.5, 1H), 3.96 (d, J=11.5, 1H), 2.78 (dd(br), J=5.5, 16.0, 1H), 2.13 (s, 3H), 2.01 (s, 3H), 1.32 (s, 3H), 1.24 (s, 3H), 1.21 (s, 3H)); ^{13}C NMR (90 MHz, $CDCl_3$): δ205.8 (s), 171.7 (s), 169.8 (s), 137.0 (d), 136.5 (s), 135.1 (d), 119.2 (s), 72.1 (d), 65.8 (t), 58.4 (d), 55.9 (d), 53.1 (s), 46.0 (t), 40.9 (t), 38.2 (s), 34.3 (s), 26.3 (q), 20.7 (q), 20.5 (t), 20.1 (q), 19.9 (t), 18.8 (t), 17.6 (2xq).

7) A. Bax and G. Morris, J. Magn. Reson., 42, 501(1981).

8) A. Bax, R. Freeman, and G. Morris, J. Magn. Reson., 42, 164(1981).

9) A. Kumar, R.R. Ernst, and K. Wuthrich, Biochem. Biophys. Res. Commun., 95, 1(1980).

10) 5: $[\alpha]_D^{23}$ - 50° (c 0.41, MeOH), mp 209 - 214 °C; IR (KBr) 3390 cm^{-1}, low resolution EIMS: m/z (%) - 335 (M^++ 1, 22), 334 (M^+,97), 319 (27), 301 (36), 285 (100),283(57), 147 (61), 135 (60), 105 (41), 91 (61); ^1H NMR (360 MHz, CD_3OD: selected peaks, δ7.10 (d, J=1.3, 1H), 7.03 (d, J=1.3, 1H), 4.01 (d, J=11.2, 1H), 3.82 (ddd, J=4.5, 9.7, 11.2), 3.44 (d, J=11.2, 1H), 3.04 (d, J=9.7, 1H), 2.76 (dd, J=16.1, 6.0, 1H), 2.15 (d, J=12.5, 1H), 2.14 (m, 1H), 1.22 (s, 3H), 1.19 (s, 3H), 0.98 (s, 3H); ^{13}C NMR (90 MHz, CD_3OD): δ138.4 (s), 138.0 (d), 136.3 (d), 120.7 (s), 85.8 (d), 69.6 (d), 66.0 (t), 57.9 (d), 57.5 (d), 47.8 (t), 44.6 (s), 42.6 (t), 39.4 (s), 35.5 (s), 26.6 (q), 23.7 (q) 21.6 (t), 20.1 (t), 19.8 (t), 18.3 (q).
6: $[\alpha]_D^{20}$ - 50° (c 0.09, MeOH), mp 242 - 245 °C; IR (KBr) 3390 cm^{-1}, low resolution EIMS: m/z (%) - 335 (23), 334 (100), 319 (29), 301(47), 283 (71), 147 (59), 135 (90), 105 (34), 91 (49); ^1H NMR (360 MHz, CD_3OD): selected peaks - δ7.09 (d, J=1.2, 1H), 7.03 (br.s, 1H), 3.87 (ddd, J=3.0, 4.3, 12.1, 1H), 2.75 (dd, J=6.0, 16.0, 1H), 2.44 (m, 1H), 2.13 (m, 1H), 1.19 (s, 3H), 1.08 (s, 3H), 0.95 (s, 3H); ^{13}C NMR (90 MHz,CD_3OD): δ138.6 (s), 138.0 (d), 136.3 (d), 120.8 (s), 74.6 (d), 67.0 (d), 65.7 (t), 57.8 (d), 50.4 (d), 45.7 (s), 42.8 (tx2), 39.5 (s), 35.6 (s), 26.8 (q), 24.5 (q), 21.7 (t), 19.7 (t), 19.6 (t), 18.3 (q).

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